

The Feasibility of Using Spent Sulfidic Caustic as Alternative Sulfur and Alkalinity Sources in Autotrophic Denitrification

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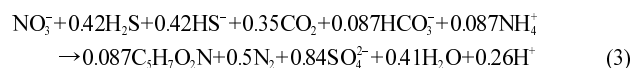
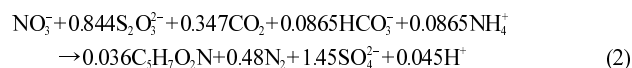
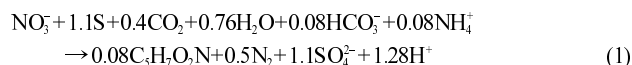
Abstract—Batch experiments using acclimated sludge to sulfur utilizing autotrophic denitrification were performed to determine the applicability of spent sulfidic caustic in autotrophic denitrification as alternative sulfur and alkalinity sources. Fluorescence *in situ* hybridization (FISH) analysis showed that the microbial community of β -proteobacteria/Eubacteria increased from 45% to 69% during enrichment period and nitrate removal reached up to 84% under this enriched sludge condition. In thiosulfate utilizing autotrophic denitrification, the initial condition at a sulfur/nitrate (S/N) ratio of 1.5 showed higher nitrate removal with 95.9%, and nitrate removal could be expressed by a first-order function of biomass concentration if all parameters such as pH, alkalinity and S/N ratio were in the optimum range. In spent sulfidic caustic utilizing autotrophic denitrification, the sulfate formation ratios to nitrate reduction were lower than those in thiosulfate utilizing autotrophic denitrification with a range of 2.65 to 2.78, and nitrate removal was over 95% at 1.0 and 1.5 S/N ratios. For S/N ratios of 1.0 and 1.5, initial alkalinities were sufficient to maintain optimum pH range of autotrophic denitrification. Furthermore, well enriched seeding sludge showed good activity of autotrophic denitrification at pH over 10. Therefore, spent sulfidic caustic could be effectively applied to autotrophic denitrification as an alternative sulfur source and an alkalinity source.

Key words: Autotrophic Denitrification, FISH, Microbial Community, Spent-sulfuric Caustic, S/N Ratio, Nitrate Removal, Sulfate Production

INTRODUCTION

The conventional biological nutrient removal (BNR) process has adopted heterotrophic denitrification mechanism requiring organic carbon sources as an electron donor [Park et al., 1999]. And it is necessary to add an exogenous organic carbon source in order to complete denitrification or to enhance the microbial activity under anoxic condition with zeolite-like material for wastewater having low COD/nitrogen ratio [Park et al., 2003]. Methanol is generally used as an exogenous carbon source, but this commercial material increases the operating costs of treatment facilities. Therefore, many researchers have tried to develop alternative carbon sources using industrial wastewater, sewage sludge, food waste, night soil and molasses, but the application of each material to real plants has only partially succeeded because of their low carbon content or instability of supply or hazardous constituents [Quan et al., 2005].

Sulfur utilizing autotrophic denitrification does not need an organic carbon source to remove nitrate and nitrite. Autotrophic denitrification oxidizes the reduced sulfur compounds such as sulfur particle, sulfide and thiosulfate while reducing NO_x to nitrogen gas by consumption of alkalinity. In this process, commercial sulfur particles and limestone have been generally used as a sulfur source and an alkalinity source, respectively. Autotrophic denitrifying reactions using reduced sulfur compounds are represented by the following equations [Bisogni and Driscoll, 1977; Batchelor and Lawrence, 1978].



As an electron donor, sulfur particles gave much lower specific denitrification rate than soluble sulfide or thiosulfate during autotrophic denitrification [Claus and Kutzner, 1985; Koenig and Liu, 2001]. However, sulfur particles have been generally used as electron donors due to their relative reliability for steady state operation of sulfur utilizing autotrophic denitrification and cost-effectiveness [Flere and Zhang, 1999]. It was also because there were no researches on alternative materials for electron donor and alkalinity of autotrophic denitrification process.

Caustic (NaOH) solutions are generally used to remove hydrogen sulfide and organic sulfur compounds by absorption mechanism in the petroleum refining industry. These spent solutions are named as spent sulfidic caustic, and these solutions typically show sulfide concentration over 2-3 wt%, pH value >12 and high concentration of alkalinity [Sipma et al., 2004]. Spent sulfidic caustics were classified as D003 (reactive sulfide) hazardous waste by U.S. RCRA (Resource Conservation and Recovery Act) and were generally incinerated for treatment and disposal. There was no attempt to reuse spent sulfidic caustic as a resource. However, the high sulfide and alkalinity of spent sulfidic caustic are expected to substitute for commercial sulfur particles and limestone, which have been generally

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Table 1. The characteristics of mixed spent sulfidic caustic

							(mg/L)	
pH	Temp.(°C)	TCOD/SCOD		TOC	H ₂ S	Alkalinity	NH ₄ ⁺ -N	
12.2	65	30,000/29,000		1,100	16,700	128,000	3,500	
As	Cd	Cu	Ni	Pb	Zn	Ca ²⁺	K ⁺	Na ⁺
N.D.	N.D.	0.12	N.D.	N.D.	0.18	1.14	40.26	23,750

N.D. is not detected.

used in autotrophic denitrification. And soluble sulfide of spent sulfidic caustic is also expected to enhance the specific denitrification rate more than conventional particle sulfur [Claus and Kutzner, 1985; Koenig and Liu, 2001].

The objective of this study was to determine the applicability of spent sulfidic caustic to autotrophic denitrification as alternative sulfur and alkalinity sources for enrichment of *Thiobacillus denitrificans*. Therefore, batch tests of spent sulfidic caustic utilizing autotrophic denitrification were conducted, and the change of microbial community during the process was observed.

MATERIALS AND METHODS

1. Characteristics of Spent Sulfidic Caustic

Two kinds of spent sulfidic caustics generated from the naphtha cracking process and ethylene producing process of S company were mixed and used as alternative material for sulfur and alkalinity source in the autotrophic denitrification process. The characteristics of mixed spent sulfidic caustic were analyzed to quantify sulfide, alkalinity, COD, heavy metal and ionic materials. The results are presented in Table 1. Sulfide and alkalinity concentrations were higher than 16,000 mg/L and 120,000 mg/L, respectively. The pH was over 12. And it was also found that any heavy metals and ionic materials were not exceeding the inhibition concentration.

2. Enrichment of Autotrophic Denitrifiers

Seeding cultures of sulfur utilizing denitrifiers were obtained from anaerobic digester of municipal sewage treatment plant (MSTP) in Busan, Korea. Obtained cultures were seeded in an upflow anoxic cylindrical continuous reactor having an effective volume of 5 L and enriched with a medium containing 1.0 g/L of KNO₃, 10.0 g/L of Na₂S₂O₅·5H₂O, 3.0 g of NaHCO₃ and other mineral nutrients. The

completion of enrichment for sulfur utilizing autotrophic denitrification condition was determined by sufficient nitrate removal and sulfate formation. The change of microbial community was also observed with FISH by a probe of EUB338 and BET42a to evaluate autotrophic enrichment [Lee et al., 2003].

3. Batch Tests of Autotrophic Denitrification

Batch experiments were performed to evaluate the applicability of spent sulfidic caustic to sulfur utilizing autotrophic denitrification as alternative sulfur and alkalinity sources. Batch reactors (300 ml Erlenmeyer flasks) were equipped with tubes to extract sample and U-shaped gas collectors partially filled with an acidic salt solution [(200 g Na₂SO₄+800 ml distilled water)+30 ml H₂SO₄] to prevent the adsorption of carbon dioxide to effluent gas. Experimental conditions are presented in Table 2; the initial volume of enriched sludge and cultivation medium for each reactor was 50 ml and 200 ml, respectively. Experiments were done three times. Initial concentration of each reactor was determined by analysis of sample extracted directly after the filling and mixing of medium and seeding sludge.

4. Fluorescence in situ Hybridization (FISH) Experiment

Sludge samples were obtained from the reactor acclimated to autotrophic denitrification and centrifuged to extract genomic DNA with DNA extraction kit (Mobio Ltd. Co., CA, USA). Probes were labeled with fluorescein isothiocyanate (FITC, Boehringer Mannheim, Germany) or tetramethylrhodamine-5-isothiocyanate (TRITC, TIBMOLBIOL, Berlin, Germany). All in situ hybridization was performed according to the procedure described by Amman [1995] in hybridization buffer (0.9 M NaCl, 20 mM Tris HCl [pH 7.2], 0.01% SDS, formamide concentration are listed in Table 3) at 46 °C for 2 hr. Subsequently, a stringent wash step was performed at 48 °C for 20 min in 50 µl of pre-warmed washing solution (0.9 M NaCl, 20 mM Tris HCl [pH 7.2], 0.01% SDS). The slides were then briefly

Table 2. Experimental conditions of batch tests and composition of each medium

Condition	M-A	M-B	M-C	S-A	S-B	S-C	S-D
Medium components	KNO ₃ , Na ₂ S ₂ O ₅ ·5H ₂ O, NaHCO ₃ , nutrients			KNO ₃ , spent sulfidic caustic ^c			
Ratio of sulfur/nitrate	0.5	1.0	1.5	0.5	1.0	1.5	2.0
NO ₃ -N (mg/L)	614	614	614	614	614	614	614
Alkalinity (mg/L as CaCO ₃)	2,600 ^a	2,600	2,600	1,090 ^b	2,180	3,270	4,360
SO ₄ -N ^c	90	90	90	60	120	180	240
Sulfur type and conc. (mg/L)	Thiosulfate (S ₂ O ₃)			Hydrogen sulfide (H ₂ S) ^d			
	470	940	1,410	140	280	420	560

^aThe theoretically required alkalinity for autotrophic denitrification of 614 mg NO₃-N/L equivalent to 2,600 mg CaCO₃/L.

^bAlkalinities of S-A, S-B, S-C are determined by the additional amount of spent sulfidic caustic.

^cSO₄-N concentration in medium originated from nutrients (M-A, M-B, M-C) and spent sulfidic caustic components (S-A, S-B, S-C).

^dSulfur compound in spent sulfidic caustic is mainly detected as hydrogen sulfide (H₂S).

^eSpent sulfidic caustic was utilized as sulfur and alkalinity source simultaneously.

Table 3. Oligonucleotide probes used in this study

Probe	Sequence (5'-3')	Specificity	Target site	FA ^a	Reference
EUB 338	GCTGCCTCCCGTAGGAGT	Eubacteria	16S (338-355)	20%	Schramm et al., 2000
BET 42a	GCCTTCCCACCTTCGTTT	β -subclass of proteobacteria	23S (1027-1043)	35%	Schramm et al., 1998

^aFA, formamide concentration in hybridization buffer.

rinsed with Milli-Q water and allowed to air dry. Slides were mounted in Prolong Gold antifade reagent (Molecular Probes, Eugene, OR). Probe-stained cells were used to detect and record by a model LSM 510 confocal laser scanning microscope (Carl Zeiss, Germany) with an Ar ion laser (488 nm) and an HeNe laser (543 nm). The oligonucleotide probes used to detect autotrophic denitrifier in this study are listed in Table 3.

5. Chemical Analyses

Alkalinity and SS (Suspended Solid) were measured by procedure from Standard Methods (20th, APHA, 1998). Nitrate, nitrite and sulfate were determined by ion chromatograph (Dionex, DX-300, USA). COD was analyzed with auto analyzer (AA3, Germany). The concentration of T-S (total sulfur) was measured by ICP atomic emission spectrometer (ICP-IRIS, Thermo Jarrell Ash, USA).

RESULTS AND DISCUSSION

1. Enrichment of Autotrophic Denitrifiers

During a 35 day enrichment period, influent NO₃-N, SO₄-N and alkalinity were maintained at 550-600 mg NO₃-N/L, 95-110 mg SO₄-S/L and 2,840-3,000 mg CaCO₃/L, respectively. After 19 days operation, effluent nitrate concentrations were maintained under 100 mg/L and nitrate removal efficiencies reached over 80%. Sulfate formation was also observed during nitrate reduction to nitrogen gas. Effluent sulfate concentrations ranged between 1,250 and 1,440 mg SO₄-S/L. Average sulfate formation to 1 mg NO₃-N (nitrate) removal was calculated as 2.94±0.12 mg SO₄²⁻-S and these values

were well matched to chemical stoichiometry of thiosulfate utilizing autotrophic denitrification. Effluent pH and alkalinities were also maintained under steady state and their values were over 7.1 and 300 mg/L, respectively. These results are summarized in Table 4.

This enrichment of autotrophic denitrification activity also could be explained through FISH analysis of microbial community. The community structure showed that BET42a-hybridized cells (autotrophs) portion in EUB338-hybridized cells (all bacteria) was increased from 45% to 69% after 35 days operation (Fig. 1). As the portion of autotrophs increased, nitrate removal efficiency was also increased.

2. S/N Ratio Effect on the Activity of Autotrophic Denitrification

Fig. 2 shows nitrate (a) and sulfate (b) profiles in a batch test to evaluate the effect of initial ratio of sulfur compound to nitrate on autotrophic denitrification process. Initial S₂O₃ concentrations were set by Eq. (2) with a stoichiometric ratio of 0.5 (insufficient ratio, M-A), 1.0 (stoichiometric ratio, M-B) and 1.5 (excessive ratio, M-C). And initial alkalinity was sufficiently supplied not to hinder the autotrophic denitrification in the reactors. It can be seen that nitrate removal proceeded according to the stoichiometric initial ratio of S₂O₃ to nitrate in Fig. 2(a). Under conditions at a ratio of 0.5 and 1.0, nitrate was removed continuously until the initial S₂O₃ converted fully to sulfate, and efficiencies of the nitrate removals were 52.7% and 94.5%, respectively. For a ratio of 1.5, nitrate removal occurred more rapidly and the autotrophic denitrification reaction was almost completed in 6 hr cultivation with 91.7% and the final efficiency was 95.9%. It is well known that sulfate production occurs while nitrate is reduced to nitrogen gas in sulfur utilizing autotrophic

Table 4. The final results of enrichment for thiosulfate utilizing autotrophic denitrification

Input	NO ₃ -N (mg/L)		SO ₄ -S (mg/L)		The ratio of SO ₄ -S formation to mg NO ₃ -N removal
	Output	Removal (%)	Input	Output	
575±25	95±5	83.5±1.5	103±7	1340±110	2.94±0.12

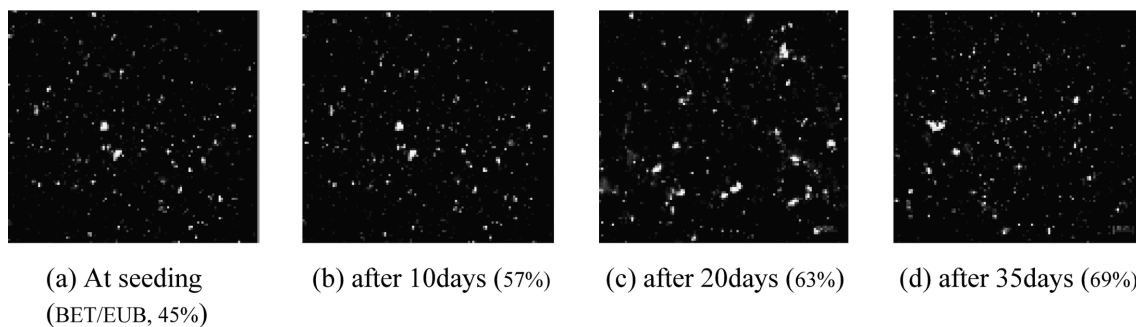


Fig. 1. The distribution and portion of β -proteobacteria containing autotrophic denitrifier such as *Thiobacillus denitrificans* in artificially enriched sludge of upflow cylindrical continuous reactor by FISH analysis: EUB338 (green), BET42a (orange), overlapped area (yellow); the scale bar on the figure indicates 5 μ m; magnification is 400 \times .

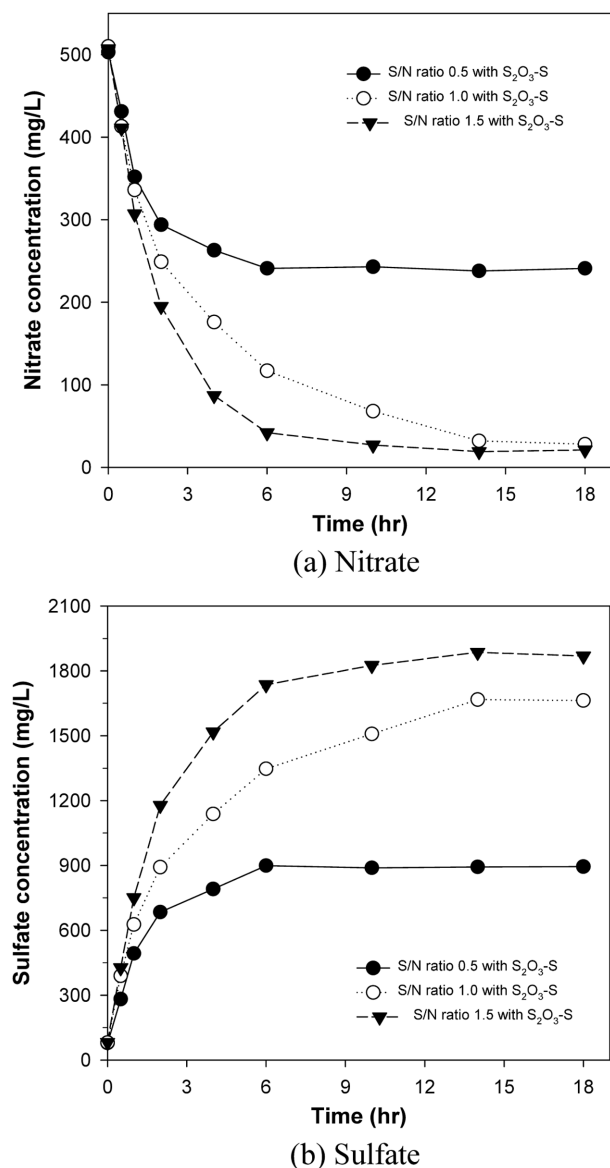


Fig. 2. Nitrate and sulfate concentration profiles during cultivation time in batch reactors at different initial sulfur/nitrate ratio using thiosulfate as sulfur source.

denitrification. Fig. 2(b) shows that sulfate formation was followed by initial S_2O_3 concentration. As the initial concentration was high according to the S/N ratio, more S_2O_3 was utilized in the nitrate reduction process. Under the condition at the ratio of 0.5, sulfate formation was stopped when the initial S_2O_3 was exhausted in 6 hr cultivation. At ratios of 1.0 and 1.5, sulfate formations were continued until nitrate removal occurred. The ratio of sulfate formation to nitrate reduction increased with an increase of the initial S/N ratio. From these results, it is considered that sulfur utilizing autotrophic denitrification is considerably affected by initial S/N ratio once sufficient alkalinity is provided. To enhance the activity of thiosulfate utilizing autotrophic denitrification, sufficient sulfur compounds need to be supplied to the reaction over stoichiometric ratio.

The following Monod model equation (Metcalf and Eddy Inc., 1991) for substrate utilization could be simplified for nitrate reduc-

tion in this thiosulfate utilizing autotrophic denitrification when thiosulfate and alkalinity are sufficiently supplied to the initial concentration of nitrate over stoichiometry.

$$-(dC/dt) = (kXC)/(K_s + C) \quad (4)$$

Where, dC/dt is nitrate reduction rate ($mg\ NO_3\text{-N/L/hr}$), k is maximum specific nitrate reduction rate ($mg\ NO_3\text{-N/g\ VSS/hr}$), C is nitrate concentration (mg/L), t is time (hr) and K_s is half-velocity constant ($mg\ NO_3\text{-N/L}$).

In sulfur-utilizing autotrophic denitrification, the half reaction constant (K_s) could be ignored in Eq. (4) because its value was known to be much less than $1\ mg\ NO_3\text{-N/L}$ [Claus et al., 1985; Batchelor and Lawrence, 2001]. And the variation of sludge concentration also could be ignored because of relatively short cultivation time for batch tests [Liu and Koenig, 2002]. From this assumption, Eq. (4) could be simplified to the following Eq. (5).

$$-(dC/dt) = kX \quad (5)$$

From Eq. (5), it is regarded that nitrate utilization rate is determined by maximum nitrate reduction rate and biomass concentration in the reactor. In Fig. 2(a), nitrate concentration at the initial S/N ratio of 1.5 decreased almost linearly with cultivation time for initial 6 hr and this result matched Eq. (5).

Fig. 3 shows alkalinity consumption and pH decrease during nitrate reduction and sulfate production. Initial alkalinities in all experiments were sufficient for complete denitrification with $2,600\ mg/L$ in the form of $NaHCO_3$. Initial pH of M-A, M-B and M-C was 8.2, 8.3 and 8.2, respectively, due to the sufficient alkalinity supply. The pH of each reactor decreased according to nitrate reduction. However, sufficient supply of alkalinity considering stoichiometric consumption maintained pH over 6.9 satisfied the optimum pH range for autotrophic denitrification. And alkalinity consumption was also observed in all experiments, and these consumptions matched to the stoichiometry of Eq. (2). The lack of alkalinity may cause a pH drop under 5.5 and nitrate reduction could be severely inhibited [Liu

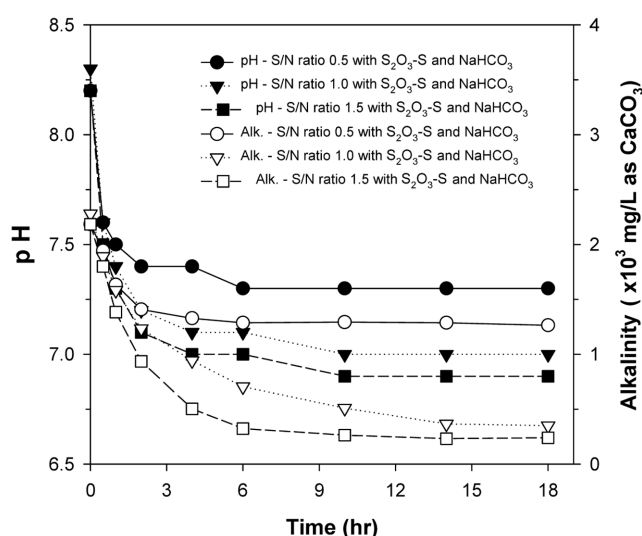


Fig. 3. Alkalinity and pH profiles during cultivation time in batch reactors at different initial sulfur/nitrate ratios using thiosulfate as sulfur source.

and Koenig, 2002]. In the M-C reactor (S/N ratio of 1.5), pH was decreased from 8.2 to 6.9 and alkalinity consumption was 1,944 mg (as CaCO_3) for nitrate reduction of 486 mg.

3. Spent Sulfidic Caustic Utilizing Autotrophic Denitrification

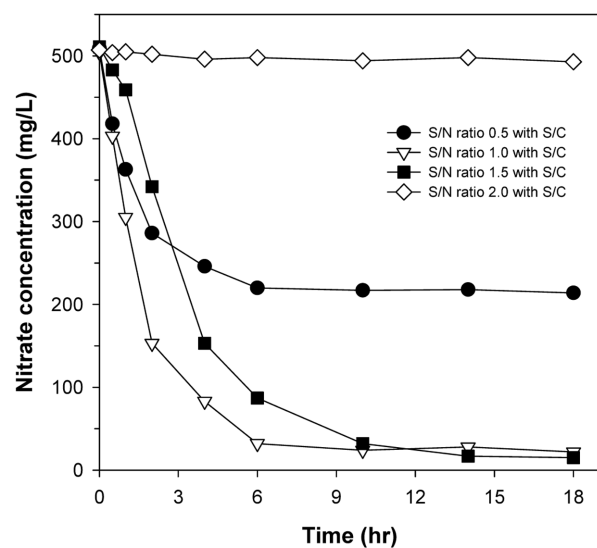
In the reactors of S-A (S/N ratio of 0.5), S-B (1.0), S-C (1.5) and S-D (2.0), spent sulfidic caustic was used as sulfur and alkalinity source instead of thiosulfate (S_2O_3) and sodium bicarbonate (NaHCO_3) which are commercial materials. Fig. 4 shows nitrate (a) and sulfate (b) profiles in these batch tests utilizing spent sulfidic caustic as alternative sulfur and alkalinity sources. The amounts of spent sulfidic caustic addition to medium were determined by the stoichiometry of Eq. (3) for initial ratio of sulfide to nitrate, since sulfide is the majority of the sulfur compounds in spent sulfidic caustic. Initial nitrate concentrations were the same conditions as those of M-A, M-B and M-C with 504-511 mg $\text{NO}_3\text{-N/L}$. However, cumu-

lative nitrate removals were higher, compared to those under the same S/N ratio conditions of thiosulfate utilizing batch tests. In each condition of S-A (0.5), S-B (1.0) and S-C (1.5), efficiencies of nitrate removal were 58.0%, 95.6% and 97.1%, respectively. It is considered that the high concentration of NaOH in spent sulfidic caustic eluted sludge to organic carbon source which could be used as an electron donor in heterotrophic denitrification. Actually, TOC concentrations were temporarily increased between the cultivation time of 1-2 hr (ratio of 0.5 and 1.0) and 2-6 hr (ratio of 1.5) (data not shown). These results could be also explained through the sulfate profiles. Under S-A and S-B conditions, sulfate was produced almost stoichiometrically during the first 1 hr cultivation time for the amount of nitrate reduction. However, sulfate production ratios to nitrate reduction were decreased due to the TOC increase which could derive simultaneous heterotrophic denitrification during sulfur utilizing autotrophic denitrification [Kim and Bae, 2000]. After 1 hr from the start-up of cultivation, sulfate production ratios to nitrate reduction decreased from 3.02 to 2.70 (S-A) and from 3.27 to 2.89 (S-B). Under the S-C condition (ratio of 1.5), nitrate reduction and sulfate production were delayed for initial period of cultivation because of high pH condition (pH 11.4). However, as the pH slowly decreased nitrate reduction and sulfate production were also recovered through simultaneous autotrophic and heterotrophic denitrification.

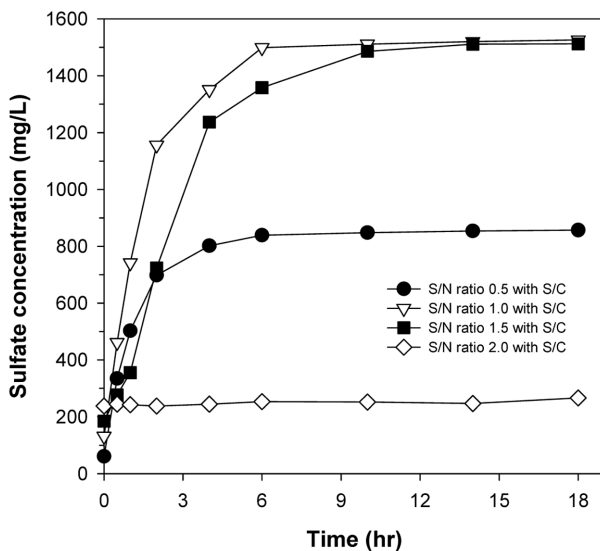
Under S-D condition (2.0), remarkable nitrate reduction or sulfate production was not observed during all cultivation time. It is considered that excessive addition of spent sulfidic caustic caused an initial pH over 11 and severely inhibited microbial activity.

From these results, a stoichiometrically optimum supply of spent sulfidic caustic as alternative sulfur and alkalinity sources could enhance the nitrate removal rate.

As shown in Fig. 4, spent sulfidic caustic supply to autotrophic denitrification should be following the stoichiometric ratio of sulfide to nitrate. Now it is needed to evaluate whether the amount of spent sulfidic caustic calculated by stoichiometric S/N ratio could be sufficient for alkalinity supply in spent sulfidic caustic utilizing



(a) Nitrate



(b) Sulfate

Fig. 4. Nitrate and sulfate concentration profiles during cultivation time in batch reactors at different initial sulfur/nitrate ratios using spent-sulfuric caustic as a sulfur source.

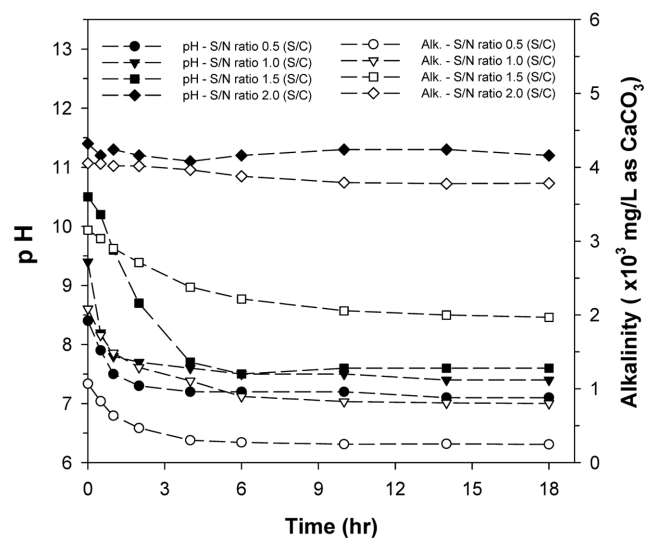


Fig. 5. Alkalinity and pH profiles during cultivation time in batch reactors using spent sulfidic caustic as a sulfur source at different initial sulfur/nitrate ratios.

autotrophic denitrification. Fig. 5 shows alkalinity consumption and pH decrease during nitrate reduction and sulfate production in spent sulfidic caustic utilizing autotrophic denitrification. Initial pH in each reactors was 8.4 (S-A), 9.4 (S-B), 10.5 (S-C) and 11.4 (S-D), respectively. These initial pH values and the differences of pH were derived from the amount of spent sulfidic caustic added. Alkalinity in each reactor was also determined by the addition of spent sulfidic caustic and the initial concentrations were 1,068, 2,084, 3,149 and 4,054 mg CaCO_3/L , respectively. As shown in Fig. 5, pH decrease and alkalinity consumption showed the same trend. However, it is remarkable that alkalinity consumptions in S-A, S-B and S-C conditions partially did not match the stoichiometry of autotrophic denitrification during the cultivation period. Alkalinity was considered partially recovered through heterotrophic denitrification, which was derived from the elution of seeding sludge by the addition of spent sulfidic caustic. For the S/N ratio of 1.0 and 1.5 conditions, initial alkalinities were sufficient to maintain the optimum pH range of autotrophic denitrification. Furthermore, well enriched seeding sludge showed good activity of autotrophic denitrification at pH over 10. Consequently, spent sulfidic caustic could be applied to sulfur utilizing autotrophic denitrification according to the stoichiometry of Eq. (3), and this amount of spent sulfidic caustic could also supply sufficient alkalinity to autotrophic denitrification.

4. Effect of Sulfur and Alkalinity Source on Autotrophic Denitrification

Fig. 6 shows the denitrification rate of the experiments at different sulfur/nitrate ratios using thiosulfate and sulfide (spent sulfidic caustic). The maximum denitrification rate in this experiment was observed at an initial S/N ratio of 1.0 with spent sulfidic caustic, and the value was 78.7 mg $\text{NO}_3\text{-N}/\text{L}/\text{hr}$. In elemental sulfur-utilizing autotrophic denitrification, Flere [1999] obtained a maximum denitrification rate of 16 mg $\text{NO}_3\text{-N}/\text{L}/\text{hr}$. The maximum denitrification rate in each experiment condition could be verified due to the biomass concentration and microbial activity as previously discussed in Eq. (5). However, the denitrification rate of our study using spent

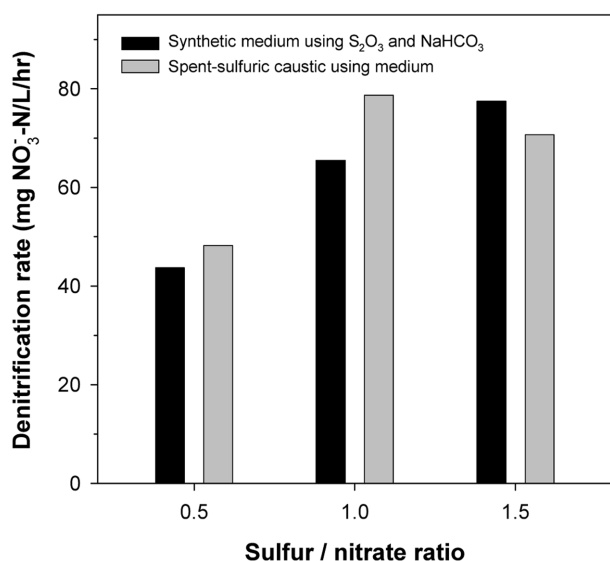


Fig. 6. Denitrification rates during initial 6 hr cultivation time at the different ratios of sulfur to nitrate.

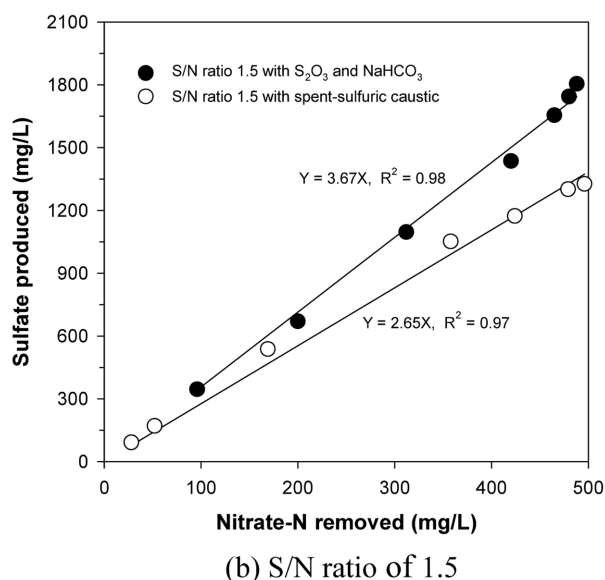
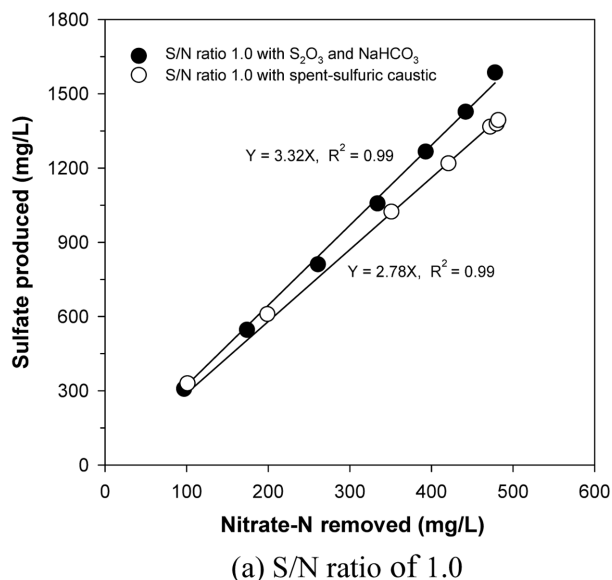


Fig. 7. Relationship of nitrate reduction and sulfate production in batch reactors at initial S/N ratios of 1.0 and 1.5 according to different sulfur sources.

sulfidic caustic proved that spent sulfidic caustic could be adopted for the autotrophic denitrification process as sulfur and alkalinity source.

Fig. 7 presents the relationship between average values of nitrate removal and sulfate production according to sulfur sources at the initial S/N ratio of 1.0 and 1.5 which showed higher nitrate removal efficiencies. While 1 mg of $\text{NO}_3\text{-N}$ was reduced, 3.32 mg of $\text{SO}_4\text{-S}$ was produced in thiosulfate utilizing process and 2.78 mg of $\text{SO}_4\text{-S}$ was produced in sulfide (spent sulfidic caustic) utilizing process at the S/N ratio of 1.0. At an S/N ratio of 1.5, 3.67 and 2.65 mg $\text{SO}_4\text{-S}$ was produced per 1 mg of $\text{NO}_3\text{-N}$ reduced under each thiosulfate and spent sulfidic caustic utilizing condition. Zhang and Lampe [1999] reported that elemental sulfur utilizing autotrophic denitrification produced approximately 3.4 g of $\text{SO}_4\text{-S}$ per 1 g of $\text{NO}_3\text{-N}$ was removed, and they achieved a nitrate-nitrogen removal efficiency of over 90% in batch experiments. Kim et al. [2004] also used ele-

mental sulfur as an electron donor in autotrophic denitrification and showed that about 4 g of sulfate was produced for every 1 g of nitrate removal in continuous operation of a pilot-scale reactor. In this pilot-scale reactor, the maximum nitrate removal efficiency was also over 90%.

From these results, spent sulfidic caustic could be effectively adopted for the autotrophic denitrification process as alternative sulfur and alkalinity sources. It is also expected that spent sulfidic caustic supply enhances nitrate removal efficiency and reduces sulfate production as well.

CONCLUSIONS

This study was performed to evaluate the feasibility of spent sulfidic caustic utilizing autotrophic denitrification and the following conclusions were obtained.

The enrichment of autotrophic denitrifier with anaerobic sludge was successfully performed during 35 days continuous operation. The efficiency of nitrate removal reached 84%, and FISH analysis showed that the microbial community in the reactor was changed to autotrophic denitrification condition. The percentage of β -proteobacteria/Eubacteria was increased from 45% to 69%.

The nitrate removal in autotrophic denitrification is dependent on the initial sulfur compound ratio to nitrate when the alkalinity is present sufficiently and pH is in the optimum range. It is also identified that nitrate removal rate is a first order function of biomass concentration if all the parameters such as pH, alkalinity, S/N ratio are satisfactorily in the optimum range.

In spent sulfidic caustic utilizing autotrophic denitrification, no inhibition effect was observed and the optimum initial S/N ratio for nitrate removal was 1.0. Sufficient nitrate removal was also observed at higher pH condition over 10, though having delay time for initial cultivation period. And it is considered that heterotrophic denitrification was partially occurring in the case of spent sulfidic caustic utilizing autotrophic denitrification due to the elution of seeded sludge.

In spent sulfidic caustic utilizing autotrophic denitrification, the sulfate formation ratios to nitrate reduction were lower than those in thiosulfate utilizing autotrophic denitrification with the range of 2.65 to 2.78, and the efficiency of nitrate removal was over 95% at the S/N ratios of 1.0 and 1.5. Therefore, spent sulfidic caustic could be effectively applied to autotrophic denitrification as an alternative sulfur source and alkalinity source.

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REFERENCES

APHA, *Standard Methods for the Examination of Water and Wastewa-*

- ter*, 20th Edition, Washington DC (1998).
- Batchelor, B. and Lawrence, A. W., "Autotrophic Denitrification Using Elemental Sulfur," *J. WPCF*, **August**, 1986-2001 (1978).
- Bisogni, J. J. Jr. and Discoll, C. T. Jr., "Denitrification using Thiosulfate and Sulfide," *Journal of the Environmental Engineering Division*, **August**, 593 (1977).
- Claus, G. and Kutzner, H. J., "Physiology and Kinetics of Autotrophic Denitrification by *Thiobacillus denitrificans*," *Appl. Microbiol. Biotechnol.*, **22**, 283 (1985).
- Flore, J. M. and Zhang, T. C., "Nitrate Removal with Sulfur-limestone Autotrophic Denitrification Processes," *Journal of Environmental Engineering*, **125**(8), 721 (1999).
- Kim, E. W. and Bae, J. H., "Alkalinity Requirements and the Possibility of Simultaneous Heterotrophic Denitrification During Sulfur-utilizing Autotrophic Denitrification," *Wat. Sci. Tech.*, **42**(3-4), 233 (2000).
- Kim, I. S., Kim, S. Y., Kim, M. S., Park, W. S. and Jung, H. J., "Novel Nitrogen Control Process Dominated by Autotrophic Denitrifying Bacteria for the Treatment of Municipal and Industrial Wastewaters," *Journal of Korean Society on Water Quality*, **1**, 67 (2004).
- Koenig, A. and Liu, L. H., "Kinetic Model of Autotrophic Denitrification in Sulfur Packed Bed Reactor," *Wat. Res.*, **35**(8), 1969 (2001).
- Lee, J. W., Lee, H. W., Lee, S. Y., Kwon, S. Y., Choi, E. S. and Park, Y. K., "Characteristics of Biological Nitrogen Removal Considering Autotrophic Denitrification and its Microbial Community," *J. of KSEE*, **25**(11), 1352 (2003).
- Liu, L. H. and Koenig, A., "Use of Limestone for pH Control in Autotrophic Denitrification: Batch Experiments," *Process Biochemistry*, **37**, 885 (2002).
- Metcalf and Eddy Inc., *Wastewater Engineering: Treatment, Disposal, Reuse*, 3rd revised ed., Revised by Tchobanoglous G, Burton FL. New York, McGraw-Hill, 371 (1991).
- Quan, Z. X., Jin, Y. S., Yin, C. R., Lee, J. J. and Lee, S. T., *Hydrolyzed Molasses as an External Carbon Source in Biological Nitrogen Removal*, Bioresource Technology, adapted 17 December (2005).
- Park, S. J., Kim, C. G., Yoon, T. I. and Kim, D. W., "Evaluation of Increased Denitrification in an Anoxic Activated Sludge using Zeolite," *Korean J. Chem. Eng.*, **20**, 492 (2003).
- Park, T. J., Lee, J. H. and Nam, H. U., "Removal of Nitrogen and Phosphorus using a New Biofilm Process-INRS (Innovative Nutrient Removal System)," *Korean J. Chem. Eng.*, **16**, 303 (1999).
- Schramm, A., de Beer, D., Wagner, M. and Amann, R., "Identification and Activities *in situ* of *Nitrosospira* and *Nitrospira* spp. As Dominant Populations in a Nitrifying Fluidized Bed Reactor," *Applied and Environmental Microbiology*, **64**(9), 3480 (1998).
- Schramm, A., de Beer, D., Gieseke, A. and Amann, R., "Microenvironments and Distribution of Nitrifying Bacteria in a Membrane-bound Biofilm," *Environmental Microbiology*, **2**(6), 680 (2000).
- Sipma, J., Svitelskaya, A., Mark, B., Pol, L. W. H., Lettinga, G., Buisman, G. J. N. and Janssen, A. J. H., "Potentials of Biological Oxidation Processes for the Treatment of Spent Sulfidic Caustics Containing Thiols," *Wat. Res.*, **38**, 4331 (2004).
- Zhang, T. C. and Lampe, D. G., "Sulfur:limestone Autotrophic Denitrification Processes for Treatment of Nitrate-contaminated Water: Batch Experiments," *Wat. Res.*, **33**(3), 599 (1999).